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(54) Title: DETECTION SYSTEM

(57) Abstract

An apparatus for automatically monitoring the presence in a fluid of an analyte includes a non-conductive solid-phase support that comprises on a surface a capture substance having specificity for the analyte. The support is movable past an opening on a housing that permits a portion of the support to be exposed to an environment that may contain the analyte. A tagging means specifically attaches a tagged second substance to the analyte that has been specifically bound to the capture substance. A detector means detects the tag attached to the tagged substance. Signaling means provide an audible, visual, or electronic signal when the level of analyte bound to the support exceeds a selected level. A plurality of analytes can simultaneously be detected continously or intermittently.

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DETECTION SYSTEM

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of provisional patent application number 60/035,788, filed January 8, 1997, entitled "Continuous Monitering (sic) System for the Detection of Air or Water Pathogens or Toxins." This application also claims the benefit of provisional patent application number 60/035,812, filed January 8, 1997, entitled "Method for Increasing the Sensitivity and Eliminating the 'Silver Step' in a Resistive Immunodiagnostic Technology."

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

BACKGROUND OF THE INVENTION

The present invention relates to an assay system for detecting chemical and biological analytes. A number of continuous monitoring systems that employ immunological detection are known, but none provides a convenient system for continuously or intermittently monitoring a plurality of analytes in a non-competitive assay. Moreover, none provides a simple, reliable and inexpensive means for archiving the data obtained. The art is desirous of an instrument that can continually or periodically monitor an environmental liquid or gas for the presence of one or more analytes.

US Patent No. 5,281,539 discloses a process for detecting organic molecular analytes in a competitive binding assay that uses the concept of a conjugate of the analyte and a signal generating molecule that is dislocated from first binding means when placed in the presence of unconjugated analyte in a fluid. The dislocated conjugate with signal generating means is then bound to second binding means whereupon a detectable signal is measured to ascertain the presence of the analyte in the fluid.

A device for carrying out the process is also disclosed.

US Patent No. 5,045,479 discloses a continuous flow competitive assay system that measures a tagged immunochemical discharged from the reactor.

US Patent No. 4,277,560 discloses a method and apparatus for immunoassay of an analyte in a flowing stream. The method of the '560 patent also relies upon a competitive equilibrium between the analyte and a complexed analyte, wherein the complexed analyte includes a detectable portion that can be converted to a measurable product whose concentration is in proportion to the concentration of the analyte in the original sample.

US Patent No. 5,183,740 also discloses a competitive displacement assay and apparatus for same.

US Patent No. 4,053,284 discloses a test apparatus for continuous flow testing of biological samples. The test apparatus includes a reaction vessel and washing means with a vacuum conduit in a single device. The apparatus can be used for competitive- or sandwich-type assays.

US Patent No. Re. 30,562 discloses an immunological testing device for simultaneously preparing a plurality of separate samples for immunoassay.

BRIEF SUMMARY OF THE INVENTION

The present invention is summarized in that an apparatus for detecting one or more analytes in an environmental fluid includes a solid-phase movable support provided within a housing having an opening therethrough for receiving an environmental fluid containing the analyte. The support is prepared by irreversibly binding thereto one or more capture substances that can bind specifically to the analyte or analytes. The capture substances can be provided in one or more separate portions (or "channels") on the support. The capture substances are selected for their ability to specifically bind to ("capture") an environmental analyte. The capture substances are preferably antibodies having a specificity for an environmental analyte of interest, or

antigens for detecting antigen-specific antibodies in the environmental fluid.

The support is movable past the opening to permit a portion of the support to be exposed to the environmental fluid at the opening for a selected interval of time, the support being movable at a rate that is sufficient to permit the analyte, if present, to become specifically and non-competitively bound to the capture substance on the support. An optional washing means is in fluid contact with the support for removing non-specifically bound material from the exposed portion of the support without removing the specifically bound analyte. A tagging means binds a detectable tag substance to the specifically-bound analyte after washing. An drying means is optionally provided for drying the support after tagging and before analysis. A detector means detects the bound tag substance. Signaling means in electrical communication with the detector means provides a detectable signal when the detector means detect that the analyte is present at or above a selected level.

It is an object of the present invention to provide an apparatus and method for continuous or intermittent detection of an analyte in an environmental fluid.

It is another object of the invention to permit simultaneous detection and monitoring of a plurality of analytes.

It is yet another object of the present invention to provide a support that can be archived after processing.

It is still another object of the present invention to provide short-term or long-term ambient sensing and monitoring of air and groundwater.

It is an advantage of the present apparatus that detection can proceed continuously or intermittently without human intervention.

It is another advantage of the present invention that the apparatus can be portable.

It is still another advantage of the present invention that the apparatus can be adapted for remote data transmission,

thus facilitating collection, processing and dissemination of data from a remote source.

Other objects, advantages, and features of the present invention will become apparent upon consideration of the following detailed description taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 is a schematic depiction of the apparatus of the invention.

Figures 2A-2D depict preferred embodiments of the support.

Figure 3 depicts a preferred detection system for use in the apparatus wherein a plurality of probes are provided.

Figure 4 depicts a preferred detection system for use in the apparatus wherein a single probe that moves among positions is provided.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an automated system for detecting analytes in an environmental fluid, which can be a liquid or a gas. The fluid is typically air or water, but can also include a biological fluid such as urine or blood, another commercial fluid(e.g., milk or juice), or a waste stream (e.g., an aqueous liquid used to wash meat products). The fluid can be an aerosol. The analyte can be any biological organism or molecule therefrom, or any chemical, molecule or other material that can be specifically captured onto a support and detected in accordance with the invention. The analytes are typically captured onto the support by specific binding to a capture substance provided on the support. If the capture substance is an antibody, the analyte can be thought of as an antigen. Typical analytes can include pathogens such as bacteria, including but not limited to an *E. coli*, *Cryptosporidium*, *Bacillus anthracis*, and *Giardia*, and air- or liquid-borne viruses and toxins produced by pathogens, including, but not limited to botulinum toxin. The analyte can also be an organic molecule such as a nerve agent, a pesticide, insecticide or

herbicide, a protein, a hormone, an allergen or the like. An example of such a molecule is Agent Orange.

The invention preferably employs technology for binding the analyte to the capture substance as described in US Patent Numbers 4,794,089, 5,137,827, and 5,284,748 (all invented by Mroczkowski, Siegesmund and Yorde), all of which are incorporated herein by reference in their entirety. The preferred method is described, for example, in Patent Number 5,284,748 in column 6, line 6 *et seq*. In that embodiment, a capture antibody that specifically binds to an analyte of interest is bound or coupled to a diagnostic element in a predetermined amount in excess of the amount required to bind all available free analyte in the sample. The sample is added to the bound capture antibody and is allowed to react. A second, conductively labeled antibody is then added. The second antibody reacts with the analyte to form a sandwich complex that comprises first antibody-antigen-second antibody-conductive particle bound to the diagnostic element. The endpoint of the system described in the incorporated patents is determined by a drop in resistance that is proportional to the amount of analyte in the sample. The resistance decreases because the conductively-labeled second antibody forms a low resistance bridge across the diagnostic element. Further details of the two-antibody system are available in the above-noted patents. The incorporated patents envision neither the movable, flexible support of the present invention, nor the preferred detection means. Although the resistive endpoint technology is a preferred technology for use in connection with the apparatus described herein, other diagnostic detection systems are also noted below.

Although this application describes the invention in terms of an antibody capture and sandwich system, it is specifically contemplated that other means for capturing the analyte to the support and for associating the bound analyte into a detectable complex are also envisioned to fall within the scope of the present invention, as the means for specifically binding an analyte to the support, and the means for binding the analyte

to a detectable tag substance are within the ability of one skilled in the art.

Figure 1 depicts a preferred embodiment of the apparatus 10 of the present invention. The apparatus 10 includes a solid-phase movable support 12 inside a housing 14 having an opening 16 therethrough for receiving an environmental fluid containing the analyte. The environmental fluid can enter the housing through the opening 16 either passively or, to increase throughput, forcibly. Pre-treatment of the environmental fluid (e.g., concentration) before entry into the housing is also possible. The support 12 is movable past the opening 16 to permit a portion of the support 12 to be exposed to the environmental fluid at the opening for a selected interval of time, the support 12 being movable at a rate that is sufficient to permit the analyte, if present in the environment, to become specifically and non-competitively bound to the support. A tagging means 18 binds a detectable tag substance to the specifically-bound analyte. A detector means 20 detects the bound tag. Signaling means 28 in electrical or mechanical contact with the detector means 20 generate a detectable signal when the detector means indicates that the bound analyte meets or exceeds a selected level. For example, the signaling means 28 can be audible or visual or can include a continuous electronic monitor of the signal along each channel of the support. One skilled in the art will also appreciate that as the detector means 20 collect data from each channel, raw or processed data can be transmitted electronically to a remote location. Conversion of analog data to digital data is specifically envisioned. It is also specifically envisioned that the apparatus can include an uplink to a satellite for remote transmission of data to a central monitoring station. The apparatus can comprise a plurality of wheels that direct the support within the apparatus.

A washing means 22 can optionally be provided between the tagging means 18 and the detector means 20 for removing non-specifically bound material from the exposed portion of the support 12 without removing the specifically-bound analyte.

Likewise, drying means 30 can optionally be provided between the washing means 22 and the detector means 20. The drying means 30 can be active means, such as a forced air stream, or passive means, such as passive evaporation of liquid from the support. It is preferable to not include the drying means from the apparatus, since adequate measurements can generally be obtained even when the support is not dry.

The movable support 12 onto which the capture substance is irreversibly bound should be flexible and, if the capture substance is an antibody or proteinaceous antigen, is preferably at least as proteinophilic as polycarbonate. Other suitable support materials include polypropylene, polyethylene and mylar. Polyurethane is not sufficiently proteinophilic. As used herein, proteinophilicity is a measure of the amount of protein that binds to a support. The amount can readily be measured spectrophotometrically and can be expressed in absorbance units. The support can be fluid-permeable to increase contact between the environmental fluid and the capture substance. The flexible support can have a thickness comparable to flexible tapes such as standard audio cassette tapes or adhesive tape. In addition to being a tape, the support can also be a thin filament (or plurality of filaments) having the indicated properties. If the support material is not inherently sufficiently proteinophilic, it can be treated to render it attractive to protein.

A suitable support for use in the apparatus also has a high but measurable resistance (on the order of between 0.5 and 20 MOhm), if the detection assay uses the resistant endpoint technology of the above-noted patents. If the resistance of an otherwise suitable support material is too high to be measured, the resistance of the support material can be reduced by adding a conductive colloidal filler material to the support. Graphite is a preferred filler material, although carbon is also effective. For example, Kapton (E.I. Dupont de Nemours), which has a resistance of about 2 MOhm, is a suitable support having a colloidal filler material. Alternatively, the support can comprise a conductive plastic material. For the other

detection technologies, no particular electrical properties are required of the support.

The support can include a single capture substance along its length or, by providing on separate portions of the support, a plurality of capture substances each having specificity for an analyte, each substance being provided in a separate portion, or analytic domain, as shown in Fig. 2A. The capture substance or substances should be provided on a surface of the support that faces the opening in the housing, unless the support is fluid permeable, in which case the substance or substances can be provided on any surface of the support or dispersed throughout the support. The capture substance or substances can be provided on the support in one or more channels using a mechanical applicator such as a spray or a brush or a sponge. It is also advantageous to provide appropriate control channels on the support.

The number of channels available is limited only by the width of the support. Although it is preferred that the separate channels be disposed in parallel along the length of the support, the separate portions can also be provided in series such that at any given time a single portion of the support containing a capture substance having specificity for a particular analyte passes by the housing opening as shown in Fig. 2B.

If the support has sufficient width, the support can be provided with a matrix of capture substances, as is shown in Fig. 2C. The position of a capture substance in the matrix can be specified by including on the support an indexing code that can identify row (shown schematically as letters in Fig. 2C) or column (numbers in Fig. 2C) positions, or both, in the matrix. The indexing code can be recognizable to, for example, electronic indexing recognition means, such as a bar code reader, that can be optionally provided in the apparatus of the invention. This arrangement permits a multiplexed detection and provide simultaneous information about many more analytes than can be detected in other arrangements. The size of the matrix should correspond to the size of the opening so that all

members of the matrix receive comparable exposure to the environmental fluid to be tested by the apparatus. The detection limit of an apparatus employing the electrical shunt resistance technology is in the range of 10^{-15} to 10^{-18} grams.

In addition to the electrical shunt resistance technology described above, a gap bridging resistance technology can be employed to improve the probability of detecting rare analytes. A gap 36 is provided on a low-resistance conductive support 38, as shown in Fig. 2D. In the gap bridging approach, the capture substance is bound to the etch portion, but can also be bound to the entire support surface. The gap can be bridged by the tag substance that binds to the analyte. A few molecules of tagged, captured analyte are sufficient to bridge the gap between the low resistance portions of the conductive support. The detector means can detect a quantitative drop in resistance when the gap is bridged. This approach can bring the detection limit into the range of 10^{-21} to 10^{-24} grams. The gap can be provided as lithographically produced high-resistance etch on a support surface. The thin etch line can be on the order of the width of a few analyte molecules. An efficient layout for the etch is a serpentine arrangement.

The support 12 can be provided on a delivery spool 24 and a take-up spool 26 such that support 12 can be fed from the delivery spool 24 past the opening 16, through the appropriate chemistry (including tagging means 18 and washing means 22), past the detector means 20 and on to the take-up spool 26. The support 12 on the take-up spool 26 can be removed and stored after analysis for future reference. It is envisioned that the prepared support 12 and delivery and take-up reels 24, 26 can be provided as a cassette, similar in structure to a standard audio cassette, which can be labeled and stored for subsequent review. The support 12 can be protected in isolated chambers when it is not exposed to the environment at the opening.

The detector means 20 can comprise, for example, a detector for assessing whether the analyte is bound to each channel on the support, as is shown in Fig. 3. The detector is attached to a plurality of separate pairs of probes 34 in

contact with each channel on the support. Alternatively, a single pair of probes can move from channel to channel on the support and the detector can monitor the status of the channels serially, as is shown in Fig. 4. The probe or probes can contact the support, for example, at a resistor wheel 32 past which the flexible support is threaded in the apparatus. If, as in the preferred embodiment, electrical resistance is the endpoint of the assay, the detector means comprises an ohmmeter, a pair of spaced-apart, positive and negative electrical conductors attached via electrical circuit means to the ohmmeter and in contact with the support. The conductors can be electrodes, such as metal brushes in continuous contact with the support. The non-conductive support constitutes a break in the circuit. The electrodes are positioned relative to the support such that if a conductive path is formed as a result of the tagging process, then the path completes a circuit between the electrodes and the resistance drops from the pre-exposure (or baseline) level. Typically, the resistance will drop to between about 100,000 and 100 Ohm, although the absolute level will vary depending upon the baseline level and the amount of analyte in the environmental fluid. The amount of analyte detected is directly proportional to the decrease in resistance. Typically, a drop of about 5% or more from the baseline level is considered a significant indicator that the analyte has been specifically detected. The use of an ohmmeter to monitor the amount of analyte bound to a non-conductive support is described in the incorporated United States patents. The detector is in electrical communication with the signaling means described elsewhere herein.

As noted, detection means other than the resistive endpoint means are envisioned to work in the disclosed apparatus. The contact between the other detector means and the support need not be physical contact, but can be energetic contact. For example, an energy dispersive analysis (EDA) can detect the presence of an analyte. EDA relies upon a detection of secondary x-rays of various energies produced after primary x-irradiation of the support with bound, tagged analyte. A

similar detection system can be employed using an inductively-coupled plasma analyzer (ICP) in place of the x-ray system.

In an EDA detection system, a variety of different metals are separately coupled to the tag substances, each metal being coupled to a substance that is specific for a unique analyte. Any metal is suitable as a tag in the energy dispersive analysis, particularly those metals having an atomic number greater than that of sodium. Preferred metals include colloidal gold, iron, silver, chromium, copper, zinc, aluminum, and magnesium. After the tag substance is coupled to the bound analyte, and unbound second substance is removed, a source of x-rays bombards the support. An x-ray detector monitors the secondary x-rays dispersed by the primary x-ray bombardment. Peaks characteristic of a particular metal tag are indicative of the presence of the corresponding analyte. As above, the specific capture substance can be provided either in parallel or in series. If the capture substances are provided in series, all of the tag substances can be tagged with a single metal, provided that the apparatus can determine which sample is passing by the x-ray source at any given time, for example, by providing a reference signal between samples. Alternatively, if a plurality of analytes are to be monitored, the support can comprise a plurality of capture substances, since the ability to positively identify the presence of an analyte in this detection system does not necessarily depend upon the position of the analyte on the support, but rather upon the spectral output measured by the x-ray detector. Data collected by the x-ray detector can be stored, analyzed, and retransmitted in the same manner as was discussed above in connection with the ohmmeter detector.

One skilled in the art can select an appropriate capture substance or substances for use in the apparatus. Capture antibodies can be monoclonal or polyclonal antibodies having a suitable specificity for an analyte of interest. An antibody can be pre-qualified for use in the apparatus by performing standard immunological tests to determine the specificity and selectivity for the analyte.

The prepared support should be stored under suitable conditions of temperature and humidity so that the bound substance or substances do not degrade or detach from the support. Appropriate storage conditions include storage in a sealed container at 4°C if the capture and tag substances are antibodies. It is advantageous if the prepared support indicates the orientation of the capture substances so that it will be apparent to the user which analyte is being detected.

In use, the prepared support is placed into the apparatus, and the apparatus is placed into position in an environment of interest that is suspected to contain the analyte of interest such that the opening is in proximity to the environmental fluid. The support is urged toward the take-up reel by the application of force, such as mechanical or electrical force which can be initiated automatically or by remote signal. The support is moved at a rate sufficient to allow any analyte of interest present in the environmental fluid to bind specifically to the support-bound capture antibody as the support moves past the opening. The support can move past the opening at a constant rate or can be advanced periodically. If the support moves past the opening at a constant rate, the amount of analyte bound to the support at a given point will be directly proportional to the amount of analyte present in the environmental fluid at the time of its exposure to the support. A speed of between 0.05" and 1" per minute is considered an appropriate speed for most applications. Alternatively, the support can be exposed intermittently to the opening for a period of between about 1 minute and about 60 minutes or more per exposure, depending upon the anticipated concentration of the analyte in the environment. The support can then be advanced to bring the next portion into contact with the environmental fluid.

Once the support has passed the opening and any analyte present in the environmental sample has had an opportunity to bind to the capture substance or substances, the support moves into a development portion of the apparatus that includes the tagging means, and optionally the washing means. The

development portion can vary depending upon the detection scheme in use. If the detection scheme is the resistive endpoint technology of the incorporated patents, the tagging means contacts the support and exposes the bound analyte to the second substance which has previously been conductively tagged. For example, the second antibody which can be specific for the bound analyte, can be tagged with colloidal (e.g., 20 - 1000 nm) gold particles, as are described in the incorporated patents. The second antibody can be brought into contact with the bound analyte either by passing the support through a vessel containing a second antibody or by contacting the support with, for example, a sponge or spray applicator. In any event, the important outcome is that the second antibody is bound specifically to the first antibody-analyte composition. If multiple analytes are being detected, the support can be exposed to a cocktail of conductively-tagged antibodies, each of which is specific for one analyte. If the conductively-tagged antibodies do not specifically bind to more than one analyte, then multiple tagging steps can be performed in a single operation. At this point, the support can move past the optional washing means to specifically remove unbound conductively tagged antibody. The washing means can likewise include either a bath into which the support is passed, a spray that removes unbound second antibody, or a sponge that wipes away unbound material. One skilled in the art is familiar with the conditions appropriate for separating unbound antibody from bound antibody.

The incorporated patents describe a silver enhancement step, that can develop the signal formed by the conductive tag substances along the conductive bridge. Silver enhancement means, in accordance with this step, can also be included in the development portion of the apparatus. However, it has recently been determined by the applicant that the silver enhancement step can be advantageously replaced by providing in the washing means a protein-degrading material, such as a 1-10% solution of a proteinase, at a concentration that destroys proteinaceous material bound to the conductive tag to leave

only the conductive tag in place. By including this aspect, an signal is achieved at much lower cost than was previously required using the silver enhancement step.

If the protein-degrading material is employed in the washing means, it is not necessary to employ a high-resistance tape of the type described above. Instead, after the support passes the washing means, a high-resistance (e.g., 2 MOhm) workpiece is brought into direct contact with each analytic domain on the support and the resistance of the workpiece is determined. A drop in resistance measured by an ohmmeter indicates the presence of the analyte specific to that analytic domain. The high resistance workpiece, such as a pad, in electrical contact to an ohmmeter, can optionally be provided in the apparatus in place of the silver enhancement means.

The support with bound tagged antibody then passes from the development portion to a detector portion that includes the detector means that monitor whether an analyte was present in the environmental sample at the time that portion of the support passed by the opening to the environment. The detector provides output to the signaling means which produce a signal when the detector output indicate that the analyte is present at or above a selected level.

Once the support passes by the detector means, it can be stored on the take-up reel. The sandwich complexes formed by the capture and tag substances and the analyte(s) are stable and persistent. Therefore, after use, the support can be stored for extended periods for subsequent analysis or for evidentiary or confirmatory purposes.

The present invention is not intended to be limited to the embodiments described herein, but rather to encompass all such modifications and variations as fall within the scope of the appended claims.

CLAIMS

I CLAIM:

1. An apparatus for detecting the presence in a fluid of an analyte, the apparatus comprising:

a housing having an opening therethrough for receiving the fluid;

a solid-phase, flexible support in the housing, the support having bound thereto a substance that can undergo a specific binding reaction with the analyte, the support being movable past the opening to permit contact between the fluid and the support, the support being movable at a rate sufficient to permit the analyte, if present, to become specifically bound to the surface-bound substance;

tagging means in fluid communication with the support for binding a tag substance to the specifically-bound analyte; and

detector means contacting the support for detecting the tag substance bound to the specifically-bound analyte.

2. An apparatus as claimed in Claim 1 further comprising a delivery spool and a take-up spool, wherein the support is attached to the spools.

3. An apparatus as claimed in Claim 1 wherein the support is continuously movable past the opening.

4. An apparatus as claimed in Claim 1 wherein the support is intermittently movable past the opening.

5. An apparatus as claimed in Claim 1 wherein the support comprises a flexible tape.

6. The apparatus as claimed in Claim 1 wherein the support comprises a proteinophilic material.

7. The apparatus as claimed in Claim 1 wherein the surface-bound substance is selected from a group consisting of an antibody and an antigen.

8. The apparatus as claimed in Claim 1 comprising a plurality of distinct capture substances bound to the support, each of which can undergo a specific pairwise binding reaction with a distinct analyte.

9. An apparatus as claimed in Claim 1 wherein the tag substance comprises an antibody conjugated to an electrically conductive substance and the detector means comprises an ohmmeter, a pair of spaced-apart, electrical conductors attached to the ohmmeter and in contact with the support via electrical circuit means connected to each conductor.

10. An apparatus as claimed in Claim 9 wherein the electrically conductive substance is a metal selected from a group consisting of gold and silver.

11. An apparatus as claimed in Claim 1 wherein the tag substance comprises an antibody-metal conjugate.

12. An apparatus as claimed in Claim 1 further comprising a signaling means in electrical communication with the detector means.

13. An apparatus as claimed in Claim 12 wherein the signaling means is selected from a group consisting of a visual signaling means, an audible signaling means and an electronic signaling means.

14. An apparatus as claimed in Claim 1 wherein the support comprises a pair of portions having low electrical resistance separated by a gap having a high electrical resistance, the substance that can undergo a specific binding reaction being bound to the gap.

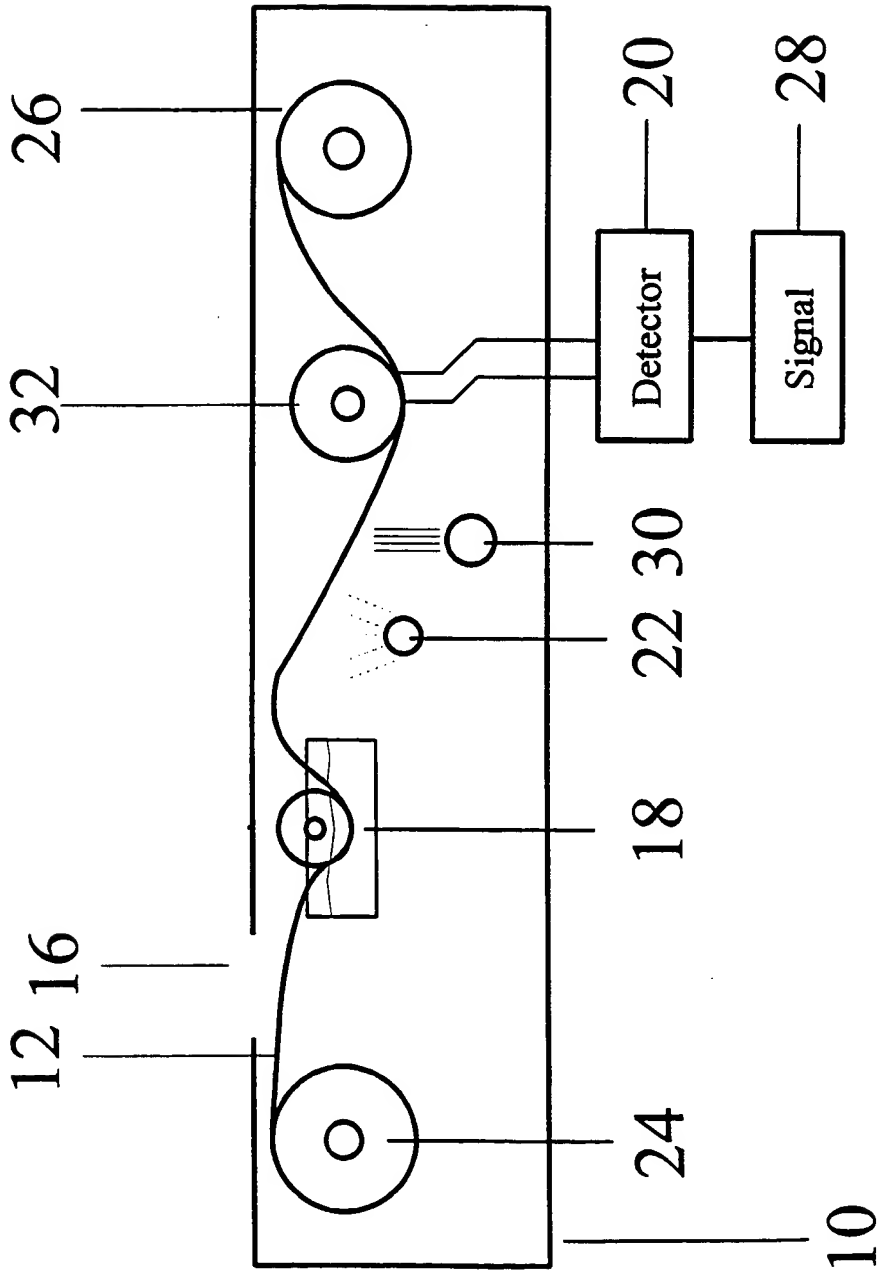

FIG 1

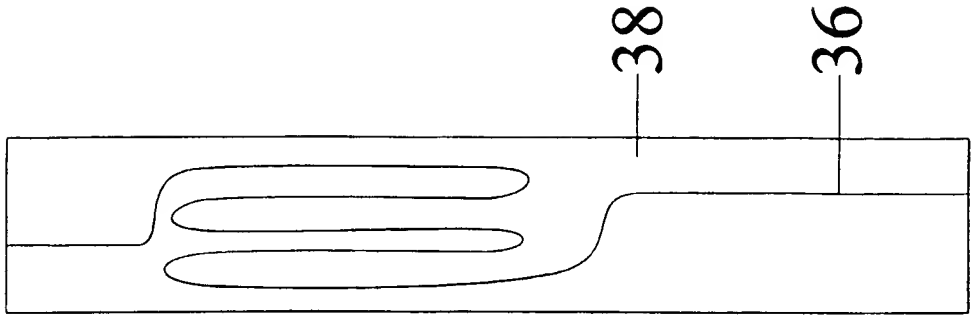

FIG 2D

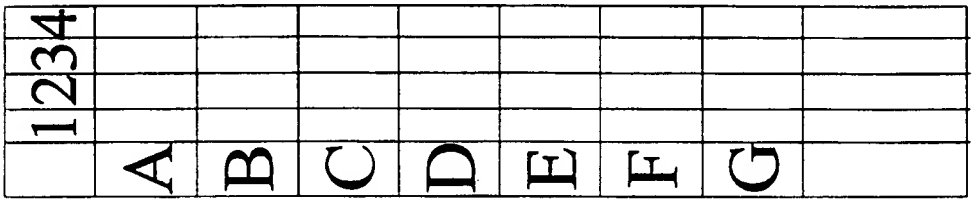

FIG 2C

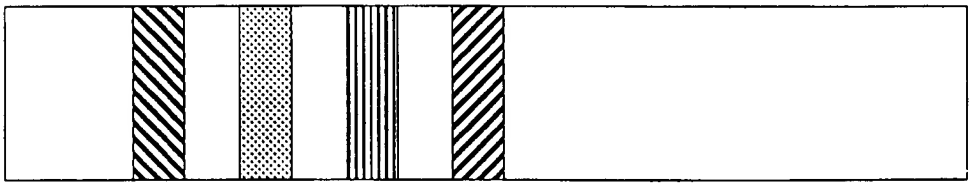

FIG 2B

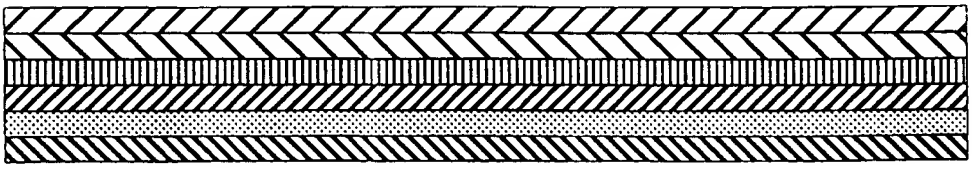

FIG 2A

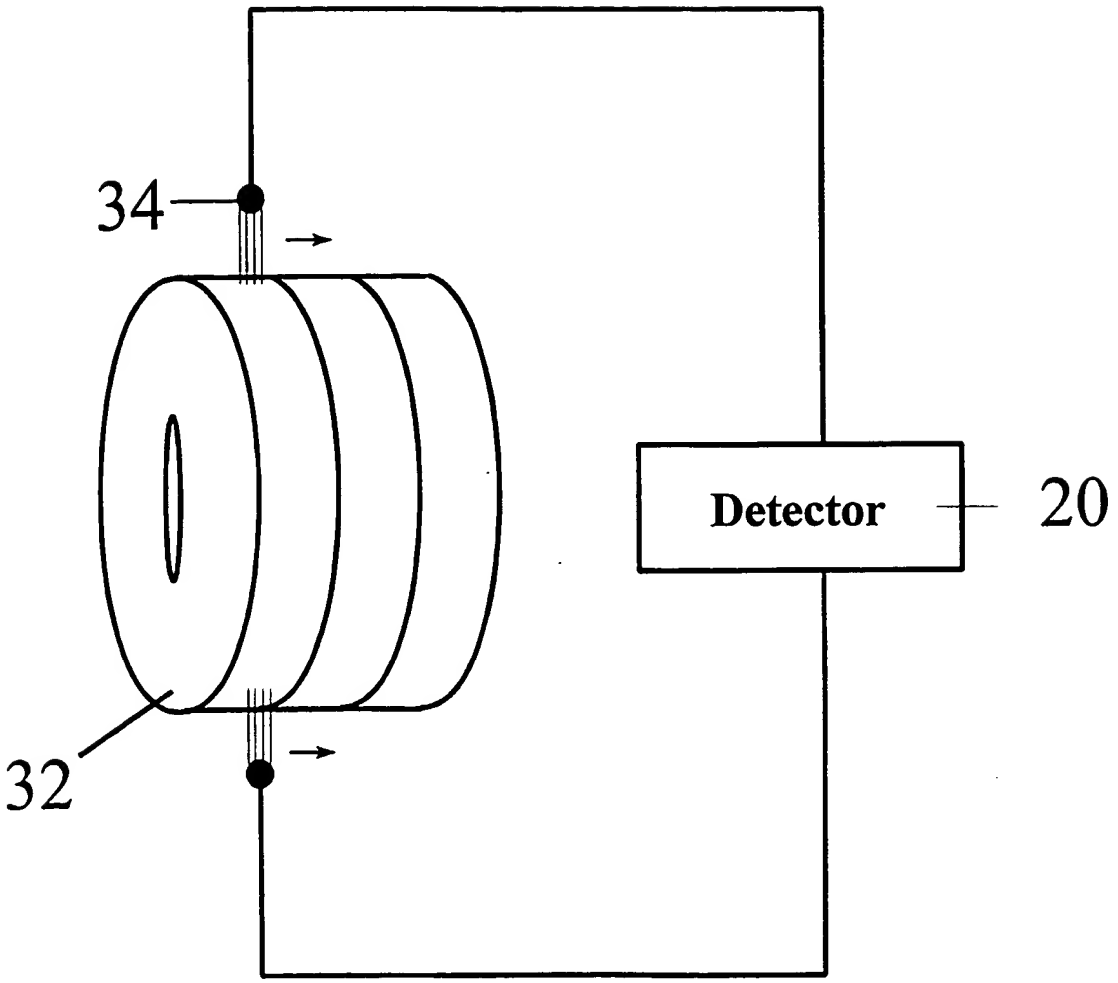

FIG 3

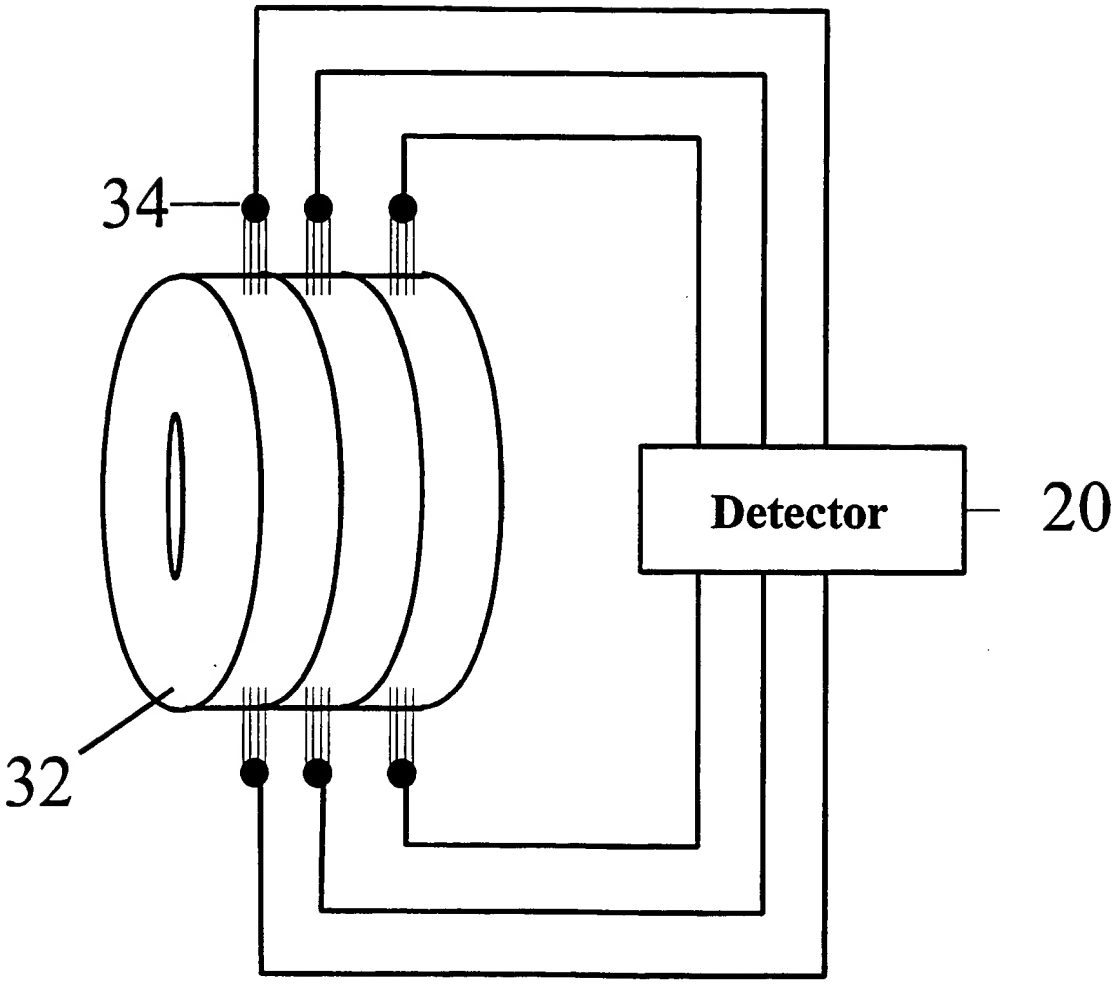

FIG 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01994

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/ 62, 63, 65, 66, 68.1; 435/7.1, 7.92, 7.93, 7.94, 7/95, 287.3, 287.6; 436/518, 528, 530, 541

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, PALM, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,679,311 A (HARTTIG et al) 21 October 1997, see entire document.	1-14
Y	US 5,672,512 A (SHAW) 30 September 1997, see entire document.	1-14
Y	US 5,362,445 A (MIYAHARA et al) 08 November 1994, see entire document.	1-14
Y	US 5,178,835 A (UEKUSA et al) 12 January 1993, see entire document.	1-14
Y	US 5,169,600 A (ISHIZAKA et al) 08 December 1992, see entire document.	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 MARCH 1998	Date of mailing of the international search report 05 MAY 1998
Name and mailing address of the ISA US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BAO-THUY L. NGUYEN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01994

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,122,343 A (ISHIZAKA et al) 16 June 1992, see entire document.	1-14
Y	US 5,120,506 A (SAITO et al) 09 June 1992, see entire document.	1-14
Y	US 5,094,816 A (ISHIZAKA et al) 10 March 1992, see entire document.	1-14
Y	US 4,824,792 A (THORPE et al) 25 April 1989, see entire document.	1-14
Y	US 4,742,009 A (BEVERLY et al) 03 May 1988, see entire document.	1-14
Y	US 4,395,493 A (ZAHNISER et al) 26 July 1983, see entire document.	1-14
Y	US 4,349,510 A (KOLEHMAINEN et al) 14 September 1982, see entire document.	1-14
A	US 4,327,073 A (HUANG) 27 April 1982, see entire document.	1-14
A	US 4,260,581 A (SAKURADA) 07 April 1981, see entire document.	1-14
Y	US 4,218,421 A (MACK, JR. et al) 19 April 1980, see entire document.	1-14
A	US 4,133,642 A (NOSAKA et al) 09 January 1979, see entire document.	1-14
Y	US 4,071,315 A (CHATEAU) 31 January 1978, see entire document.	1-14
A	US 3,979,181 A (PLAKAS) 07 September 1976, see entire document.	1-14
A	US 3,675,488 A (VIKTORA et al) 11 July 1972, see entire document.	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01994

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

G01N 21/00, 31/22, 33/544, 33/538, 33/53, 33/567, 33/537, 33/543; C12M 1/00; C12N 1/00, 1/20

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/ 62, 63, 65, 66, 68.1; 435/7.1, 7.92, 7.93, 7.94, 7/95, 287.3, 287.6; 436/518, 528, 530, 541